Effects of Childhood Trauma, Daily Stress and Emotions on Daily Cortisol Levels in Individuals Vulnerable to Suicide

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ABSTRACT

Objectives: Suicide is a leading cause of mortality worldwide. Dysregulated hypothalamic-pituitary-adrenal axis activity, as measured by cortisol levels, has been identified as one potential risk factor. Evidence has indicated that childhood trauma is associated with dysregulated cortisol reactivity to stress in adulthood. The current study investigated for the first time whether childhood trauma and daily stressors and emotions were associated with diurnal cortisol levels over a 7-day study in individuals vulnerable to suicide. *Methods*: 142 participants were categorized according to their suicidal history into three groups: suicide attempt, suicidal ideation or control group. Participants completed questionnaires before commencing a 7-day study. Cortisol samples were provided immediately upon waking, at 15 mins, 30 mins, 45 mins, 3 hours, 6 hours, 9 hours and 12 hours on 7 consecutive days. Measures of daily stressors, mood, defeat and entrapment were completed at the end of each day. *Results*: Participants in the suicide attempt and ideation groups released significantly lower cortisol upon awakening (CAR) and had a tendency towards flatter wake-peak to 12 hour (WP-12) cortisol slopes compared to controls. Childhood trauma was found to be associated with significantly lower CAR and a tendency towards flatter WP-12 cortisol slope. Childhood trauma also had an indirect effect on suicide vulnerability group membership via lower daily CAR levels. Lower CAR was associated with increased suicide ideation at 1-month but not 6-months. Daily stress and emotion measures were not associated with cortisol levels. *Conclusions*: This is the first 7-day daily diary investigation of naturally fluctuating cortisol levels in individuals vulnerable to suicide. The results indicate that dysregulated hypothalamic-pituitary-adrenal (HPA) axis activity is associated with suicidal ideation and behavior. Childhood trauma appears to be an important distal factor associated with HPA-axis dysregulation.

General Scientific Summary

Individuals vulnerable to suicide release less cortisol, the stress hormone, when they wake in the morning, and these lower levels are useful predictors of future suicidal ideation. Childhood trauma is an important determinant of lower waking cortisol and suicide risk in adulthood.

Keywords: early life adversity, stress, allostatic load, cortisol reactivity, cortisol awakening response

INTRODUCTION

Suicide is a leading cause of mortality worldwide and is a major global health issue (WHO, 2014). It is estimated that 800,000 people die by suicide each year and there are 25 million nonfatal suicide attempts annually (Centers for Disease Control and Prevention [CDC], 2016; WHO, 2014). As a result, for many decades, there has been considerable scientific effort aimed at better understanding the causes of suicidal behavior. The resulting body of work has culminated in a number of models that implicate psychological, social, psychiatric and neurobiological factors in predicting suicide risk (Mann et al., 1999; O'Connor & Kirtley, 2018; O'Connor & Nock, 2014; van Heeringen & Mann, 2014; van Orden et al., 2010). Recent work has focussed attention on the role of the hypothalamic-pituitary-adrenal (HPA) axis and the stress response system in suicidal behaviour (Giletta et al., 2015; Melhem et al., 2016; 2017; McGirr et al., 2010; O'Connor et al., 2016; 2017). Specifically, a small number of studies has investigated whether cortisol reactivity to laboratory stressors may be associated with suicide attempt and ideation.

McGirr et al. (2010) found that healthy first degree relatives of individuals who had died by suicide exhibited blunted cortisol reactivity to an acute psychosocial stressor compared to risk-free controls. These findings suggest that an inability to mount an adaptive physiological response to stress may be a biological marker for suicide risk. Moreover, O'Connor et al. (2017) also found that individuals who had made a previous suicide attempt exhibited significantly lower cortisol response to an acute stressor compared to individuals in an ideation and a control group. Moreover, participants who made an attempt within the past year exhibited the lowest cortisol response compared to participants with a more distant history of attempt. However, the extent to which they are representative of every day stress experiences or whether diurnal cortisol processes are also dysregulated in naturalistic settings in individuals vulnerable to suicide is unknown.

The diurnal pattern of cortisol production is characterised by two distinct components: the peak levels after awakening (i.e., the cortisol awakening response, CAR) and the diminishing levels throughout the rest of the day (i.e., the diurnal cortisol slope; Adam et al., 2017; Clow et al., 2004; Fries et al., 2009; Pruessner et al., 1997). Cortisol plays an important regulatory function for many of the body's basic biological systems (e.g., metabolic, immune, inflammatory processes) and disruption of its diurnal rhythm is likely to affect the functioning of these systems that may have consequences for health over time (Lupien et al., 2009; Sapolsky et al., 2000). For example, a recent meta-analysis (excluding the CAR) has shown that flatter cortisol slopes across the day are associated with poorer mental and physical health outcomes including depression, immune/inflammatory outcomes, obesity, cancer and mortality (Adam et al., 2017). Other findings have also suggested that variations in minor daily stressors can also influence day-to-day cortisol levels (Gartland et al., 2014; Adam et al., 2006). However, none of these studies have explicitly explored relations with suicidal behavior. Nevertheless, based upon these findings and the evidence from laboratory stress studies, one would hypothesize that a flatter cortisol slope across the day might be associated with vulnerability to suicide.

The CAR has also been linked with a range of health outcomes, though, the pattern of results has been mixed (e.g., Adam et al., 2006; Chida & Steptoe, 2009; Clow et al., 2010; Gartland et al., 2014; O'Connor et al., 2013). In terms of psychological stress, a number of studies have found links between stress and increases in the CAR (e.g., De Vugt et al., 2005; Wust et al., 2000). Conversely, other evidence has shown that chronic stress may disrupt HPA axis regulation and lead to a blunted CAR (e.g., Thorn et al., 2006; O'Connor et al., 2009; 2013). Moreover, a comprehensive meta-analysis, conducted by Chida and Steptoe (2009), confirmed these mixed findings and reported that different psychosocial factors are associated with both enhanced and reduced cortisol awakening response. More recently,

Boggero et al. (2017), using a combination of meta-analysis and P-curve analysis, also found divergent findings with depression being linked to higher CAR and posttraumatic stress being linked to lower CAR.

There is a growing body of evidence showing that fluctuations in within-person daily stressors are important to understanding stress-outcome processes (Almeida, 2005; Gartland *et al*., 2014; Smyth *et al*. 2018). Previous research has been overly reliant on laboratory-based and cross-sectional methodologies and has used single indices or snap-shot measurements of stress or suicide-related behaviours. Moreover, few studies have explored the day-to-day dynamics between psychological and physiological outcomes over an intensive period of time in the context of stress-cortisol-suicide relations. A critical feature of this approach is that, because stress is a process, assessments should be repeated over time. In the current study, we assessed daily stress in two ways. First, we used a modified version of the Perceived Stress Scale-Brief (Cohen, Kamarck & Mermelstein, 1983). However, we also used a free response, open-ended diary approach in order to allow respondents to record day-to-day stressors or hassles that are part of everyday life. This approach has the advantage of not constraining respondents to a limited number of different types of events and is easy to administer on a daily basis (see O'Connor & Ferguson, 2016).

In addition to measures of daily stress and mood, we were also interested in exploring the role of daily levels of defeat and entrapment on cortisol levels. Defeat and entrapment have been identified as important variables in understanding suicide. For example, the Integrated Motivational-Volitional Model (IMV; O'Connor, 2011; O'Connor & Kirtley, 2018) of suicidal behaviour provides a theoretical basis for examining the factors associated with the development of suicide ideation and the transition from ideation to suicidal behaviour (i.e., suicide attempts). The model conceptualises suicide as a behaviour that results from a complex interplay of factors; and provides a detailed map of the pathway

from ideation to behaviour, through defeat and entrapment. The IMV model proposes that the central predictor of a suicide attempt is an individual's intention to engage in suicidal behaviour. Feelings of defeat/humiliation trigger feelings of entrapment, which in turn predict intention (i.e., ideation) as a solution to life circumstances (Branley-Bell et al., 2019).

Therefore, the primary aims of the current study were to examine whether suicide vulnerability grouping (based upon history of suicide attempt or suicide ideation compared to control participants) was associated with daily CAR and cortisol levels across the rest of the day over a 7-day time window and whether daily stressors and emotions including defeat and entrapment influenced these diurnal cortisol levels.

As outlined earlier, dysregulation of HPA axis activity has been found to be associated with suicidal behavior (e.g., Melhem et al., 2016; 2017; O'Connor et al., 2016; 2017). Evidence from these studies is converging to suggest that low (or blunted) cortisol responsiveness to stress is linked to aspects of suicidal behavior in adults and that components of this dysregulation might be a heritable risk factor for suicide (McGirr et al., 2010; O'Connor et al., 2018). As such, researchers have turned their attention to seeking to understand the factors that may contribute to HPA axis dysregulation in individuals vulnerable to suicide. A strong candidate is childhood trauma. A number of studies have found childhood trauma to be associated with suicide risk, as well as with depression and psychopathology in adulthood (e.g., Carr et al., 2013; Marshall et al., 2013). For example, Marshall and colleagues (2013), in a prospective cohort study, found that severe sexual, physical and emotional childhood abuse conferred a substantial increased risk of suicide in illicit drug users.

Childhood trauma has also been linked clearly to altered dynamics of the HPA axis and to persistent sensitization of the stress response system (e.g., Carpenter et al., 2007; 2011; Gerritsen et al., 2010; Heim et al., 2000; Heim et al., 2008; Power et al., 2012). In two

studies, Carpenter showed that higher levels of childhood trauma were associated with lower cortisol reactivity to a laboratory stressor (Carpenter et al., 2007; 2011). Another study, using data from the Oklahoma Family Health Patterns Project, showed that early life adversity was linked to reduced cortisol reactivity to an acute stressor (Lovallo et al., 2013). Power et al. (2012), using data from the 1958 British birth cohort study, found evidence that childhood maltreatment was associated with flattened morning cortisol levels in mid-adulthood. Similar findings were also reported by Gerritsen et al. (2010), whereby, early life events were associated with lower cortisol in the morning and a flatter slope across the day in a large population-based study of older persons. A recent meta-analysis found that childhood maltreatment was associated with low awakening cortisol in studies incorporating more rigorous designs (i.e., agency-referred samples; Bernard et al., 2017). In the context of suicide risk, O'Connor et al. (2018) found that higher levels of childhood trauma were associated with lower resting cortisol and blunted cortisol reactivity to stress in the laboratory. However, the extent to which the distal effects of childhood trauma extend outside the laboratory and impact on HPA axis functioning in naturalistic settings is unknown. Therefore, a secondary aim of the current investigation was to test whether any observed effects of childhood trauma on membership of suicide vulnerability group were mediated by daily cortisol levels.

To summarize, a number of distal and proximal factors may account for the disparate findings observed across the different cortisol-suicide studies reviewed here including variations in: methods utilised, samples tested, age of participants, chronicity of stress, influence of daily stressors, emotions and childhood trauma history. However, in order to improve understanding of the pathways through which stress contributes to suicide, there is a need to investigate the impact of distal and proximal determinants of suicidal behavior together in the same study. Therefore, the current investigation aimed to use a 7 day daily

diary, multi-level, prospective approach that integrated distal (e.g., childhood trauma; family history) and proximal risk factors (e.g., current stress and emotions) in order to further elucidate the role of cortisol reactivity in the context of suicide vulnerability in a naturalistic setting and to explore predictive effects at 1 month and 6 month follow-up. The primary aims of the study were:

1. To examine whether suicidal vulnerability grouping (based upon history of suicide attempt or suicide ideation compared to control participants) was associated with reduced CAR and flatter cortisol levels across the rest of the day.
2. To examine the effects of childhood trauma on the CAR and cortisol levels across the rest of the day in individuals vulnerable to suicide and control participants,
3. To test whether any observed effects of childhood trauma on membership of suicide vulnerability group were mediated by daily cortisol levels.

The secondary aims were:

i) To investigate whether daily stressors and emotions were associated with cortisol levels across the day in individuals vulnerable to suicide and whether these relationships were moderated by vulnerability group.

ii) To explore whether family history of suicidal behavior was associated with CAR and cortisol levels in individuals vulnerable to suicide.

iii) To investigate whether mean cortisol levels across the study week predicted later suicidal ideation or attempt levels at 1 month and 6 month follow-up.

METHODS

Design and Participants

One hundred and fifty-four participants were recruited to a suicide attempt (n=53), a suicidal ideation but no attempt (n=52) and a control group (n=49) based upon established measures of suicidal behavior (see below). Participants were aged between 18-63 years of age (M = 27.74 years, SD = 9.27 years) and were predominantly Caucasian (see Table 1 for ethnic background breakdown). The sample consisted of 105 (68.1%) females, 49 (31.9%) males. Participants were recruited to the study in response to a local advertising campaign on websites (e.g., Gumtree, Twitter), via posters, flyers and emails. Eligible participants were required to be at least 18 years old and to understand English. Suicidal ideation and attempt were assessed using the Self-Injurious Thoughts and Behaviors Interview (SITBI; Nock et al., 2007) and the Beck Scale for Suicide Ideation (Beck et al., 1988). Participants were allocated to the suicide attempt group if they reported attempting to take their own life in the past (lifetime) or to the ideation group if they reported having thoughts of ending their life in the past 12 months (but not acting on these thoughts). Participants were recruited to a control condition if they reported no history of suicide attempt or ideation (and did not report any current psychiatric or psychological conditions). Following screening of the cortisol data, twelve participants' data were unable to be included in the statistical analysis (see Treatment of cortisol section below). Therefore, the statistical analysis was conducted on 142 participants (control group = 47, ideation group = 46, attempt group = 49; see Table 1 for baseline characteristics and demographics and Table 2 for descriptive statistics for the main study variables). In the attempt group, 14 participants reported an attempt within the previous 12 months and 35 participants reported an attempt more than 12 months ago. The range of suicide attempt methods used in the most recent attempt is shown in Table 1. In terms of family history of suicide, 25 participants reported they had a first degree relative who had

attempted or died by suicide (control group = 4 [8.5%], ideation group = 8 [17.49%], attempt group = 13 [26.5%]). Moreover, it is important to note that 5 of the 14 participants who attempted suicide in the past 12 months had a family history of suicide. At baseline, 31.7% (n=45) of participants reported using prescribed medication (control group = 6 [12.8%], ideation group = 17 [36.9%], attempt group = 22 [44.0%]).

Participants were not included in the study if they had been diagnosed with a neuroendocrine or chronic pain condition, were taking steroid-based medication, antibiotics or anti-inflammatories, were pregnant (or had recently been pregnant) or had used recreational drugs in the last month. Participants were paid £40 for completing both laboratory visits (£30 for the first visit, and £10 for the second visit). Participants also received a £10 Amazon gift voucher for each completed follow up interview. Follow up interviews were conducted by telephone or online survey at one month and six months after the second laboratory visit. The current study was approved by the Research Ethics Committee of the School of Psychology, University of Leeds and the US Department of Defense Human Research Protections Office.

The current study was designed to maximise the reliability of our main outcome measure (i.e., cortisol levels). It is now well established that there is considerable intra-individual variability in cortisol awakening responses (Stalder et al., 2016; Hellhammer et al., 2007; Almeida et al., 2009). For example, Hellhammer et al. (2007) showed that on a single day, 61-82% of the variability in cortisol awakening responses is determined by situational (or state) factors and 15-37% by trait-like factors. As a result, recent expert consensus guidelines, have recommended that cortisol assessments are made on at least six days per person in order to achieve reliable cortisol awakening response data. Therefore, in the current study, we sampled participants 8 times per day on 7 consecutive days. Moreover, based on our earlier work on stress and the cortisol awakening response with a much smaller sample

size over 4 days (n= 64, Gartland et al., 2014) and balancing recruitment practicalities of a difficult to reach vulnerable group, we were confident that the sample size of 154 (allowing for drop-out rates/technical difficulties) would be more than adequate to detect cross-level interaction effects using multilevel analyses (Snijders & Bosker, 1999).

Daily diary measures

Daily stressors. Participants completed an online diary every evening for 7 consecutive days starting the day after their laboratory visit. Using a free response format, participants were asked to report up to eight stressful events or hassles they had experienced that day. They were also asked to report the timing of the stressor, from when it began to when it ended, and rate how intense they felt the stressor was from 1 (not at all intense) to 5 (very intense). The total number of stressors reported each day acted as the main measure of daily stress.

Perceived Stress Scale-Brief (PSS-Brief; Cohen, Kamarck, & Mermelstein, 1983). This 4-item measure was amended to measure perceived stress over the day, rather than the past month. The within-person and between-person Omega reliability coefficients (Geldhof, Preacher & Zyphur, 2014) for the PSS-Brief in the current sample were 0.62 and 0.73, respectively.

Positive and Negative Affect Schedule – Short Form (PANAS-SF; Mackinnon et al., 1999). Participants were asked to indicate the degree to which they have experienced 10 feelings and emotions over the course of the day, ranging from 'very slightly or not at all' to 'extremely'. The within-person and between-person Omega reliability coefficients for the positive mood in the current sample were 0.75 and 0.77, respectively. The within-person and between-person Omega reliability coefficients for the negative mood sample were 0.76 and 0.80, respectively.

Defeat and Entrapment. Participants were also asked to rate the extent to which they have felt both 'defeated' and 'trapped' over the course of the day, ranging from 'very slightly or not at all' to 'extremely'.

In addition to the online diary completed every evening, participants also had a paper sample diary to record when each cortisol sample was due and the time the sample was taken. This was to enable the researcher to identify any samples which had been taken at the incorrect time intervals.

Completion rates for the daily diary by group. The mean completion rates for the daily diaries in each of the groups was good. The highest levels were in the control group (mean = 6.67 days, SD = 0.66, range 4 to 7, then in the ideation group (mean = 6.40 days, SD = 1.23, range 2 to 7) followed by the attempt group (mean = 6.31 days, SD = 1.29, range 1 to 7). The total number of daily diaries included in the main analyses was 798 from a maximum of 994 (representing 19.7% missing or incomplete diaries).

Mean sampling times. The mean sampling times for the control group (CG), the ideation group (IG) and the attempt group (AG) were as follows: Waking (CG = 8:01am, IG = 8:31am, AG = 8:31am), at 15 minutes (CG = 8:16 hrs, IG = 8:46 hrs, AG = 9:05 hrs), at 30 minutes (CG = 8:31 hrs, IG = 8:59 hrs, AG = 9:00 hrs), at 45 minutes (CG = 8:44 hrs, IG = 9:16 hrs, AG = 9:17 hrs), 3 hours (CG = 11:00 hrs, IG = 11:38 hrs, AG = 11:37 hrs), at 6 hours (CG = 13:53 hrs, IG = 14:33 hrs, AG = 14:41 hrs), at 9 hours (CG = 16:59 hrs, IG = 17:39 hrs, AG = 17:32 hrs), and at 12 hours (CG = 19:29 hrs, IG = 19:25 hrs, AG = 18:36 hrs).

Suicidal ideation at baseline, 1 month and 6 month follow-up. In keeping with earlier work (O'Connor et al., 2017), suicidal ideation was measured using the combination of the "wish to die" item (#2) and the "desire to kill myself" item (#4) from Beck's Scale for Suicide Ideation (SSI, Beck, Steer & Rantieri, 1988). This measure provides a clear and

unambiguous measure of suicide ideation. Cronbach's alpha for the summed scale was 0.84, 0.85 and 0.80 at baseline, 1 month and 6 months, respectively.

Background questionnaire measures

Child Trauma Questionnaire (CTQ; Bernstein et al., 2003). A brief 28-item self-report inventory was used to assess for a history of abuse or neglect in childhood or adolescence. The CTQ has five subscales relating to types of maltreatment: emotional, physical and sexual abuse and emotional and physical neglect, with five items for each subscale (1 = 'never true', 5 = 'very often true'). The total CTQ score was computed by summing each of the subscale scores. In addition, each subscale has a cut-off score to indicate a level of severity of childhood trauma ranging from none (or minimal), low (to moderate), moderate (to severe) and severe (to extreme). Therefore, for descriptive purposes, we created a total childhood trauma exposure score. For each subscale, participants who reported scores in the moderate or severe range received a score of 1 to indicate exposure to that type of trauma. The cut-off scores for each the subscales for moderate or severe were: emotional abuse > 12, physical abuse > 9, sexual abuse > 7, emotional neglect > 14, and physical neglect > 9. As a result, scores ranged on the CTQ ranged from 0 to 5. The Cronbach's alpha in the current sample ranged from 0.75 to 0.91.

Cortisol measurements

The participants provided 8 salivary samples of cortisol per day over the following 7 days (56 samples per participant in total). Cortisol samples were collected from saliva using Salivettes (Sarstedt, UK). The salivette contains a cotton dental roll inside a plastic tube, which the participant is required to place in the mouth for 30 to 45s before replacing in the tube. Participants were instructed to take the samples at the following times: immediately upon waking (when still in bed), +15 mins after waking, +30 mins, +45 mins, + 3 hours, +6

hours, + 9 hours and +12 hours. Participants were instructed to refrigerate the salivettes as soon as possible after taking each sample, to preserve sample stability as well as possible (Groschl et al., 2001). Participants were instructed to refrain from eating, drinking caffeine, alcohol, acidic drinks, smoking and brushing their teeth immediately before and during sample taking. Upon returning their samples to the laboratory on their second visit, the researcher froze all the salivary samples below -20C. The samples were then sent for assaying (packed in insulated cool boxes with ice packs) in duplicate. After defrosting and spinning, the saliva samples were assayed by Salimetrics. Cortisol levels were determined by using a competitive enzyme-linked immunosorbent assay kit (ELISA) designed for analysing saliva. Intra-assay and weighted inter-assay coefficients of variation (CV) of the assay in the current study were 5.23% and 6.04% respectively. Any samples with concentration CV% > 15% were re-analysed resulting in 48 samples being re-tested.

Treatment of cortisol data

A two stage approach was adopted. In stage 1, the number of missing data points was calculated for each time point and these ranged from 2.6% for the +15 minute samples to 5.8% for the +12 hour samples with a mean of 3.44% across all time points. Next a Little's MCAR test was performed to determine whether data were missing completely at random and found to be non-significant ($X^2 = 268.95$, $p = .28$). Therefore, the missing data were replaced using single imputation using the estimation maximisation method. In stage 2, we followed the approach adopted by Smith et al. (2018) and directly informed by Griefahn and Robens (2011), Smyth et al. (2013) and Stalder et al. (2016). All cortisol data were inspected for potential outliers using the following criteria: 1. Cortisol levels that were greater than 2.5 standard deviations from the sample mean for that time point were excluded given that they may indicate a sampling protocol violation, a technical or procedural problem with the assay or indicate ill-health (n=129 samples). 2. Samples were also excluded if the participant

reported they had provided their waking sample (+00) greater than 10 minutes after waking (n=41 samples). After exclusion of these samples, the mean difference between when the waking sample should have been taken and when it was reported to be taken was similar across the groups (CG = 0.40 min, IG = 0.63 min, AG = 0.58 min; p=0.36). In cases were issues were identified the samples for the entire day were removed from the analysis as it was likely that these data were compromised. Before conducting the statistical analyses the cortisol levels were log transformed in order to improve their skewness, however, non-transformed values are presented as the results were very similar with and without transformation. The skewness was improved post-transformation (AUCg = 2.27, WP-12 = 2.31) and inspection of the distributions indicated normality.

Cortisol awakening response (CAR) was assessed by calculating the Area Under the Curve with respect to ground (AUC_G) for the saliva samples collected immediately upon waking (0), at 15, 30 and 45 min following established procedures (Gartland et al., 2014; Pruessner et al., 2003). The AUCg provides a measure of the total amount of cortisol secreted within a specific time window. Specifically, using a standardised formula, it "calculates the total area under the curve of all the (relevant) measurements as the area of interest. It thus takes into account the difference between the single measurements from each other (i.e., the change over time) and the distance of these measures from the ground, or zero (i.e., the level at which the changes over time occur)" (Pruessner et al., 2003; p. 918). In addition, we elected to use this measure because it has been employed in comparable studies investigating the effects of chronic stress and cortisol (e.g., Chida & Steptoe, 2009) and we wanted to focus on a single measure of cortisol awakening in order to reduce the number of statistical tests performed relating to our primary outcome[1].

Wake-peak to 12 hours (WP-12) measure was calculated as the difference between the highest cortisol level from the 0, 15, 30 and 45 minute samples and the 12 hour sample.

Diurnal cortisol slope was assessed using a multi-level modelling approach similar to Ferguson (2008) where cortisol levels (at 3, 6, 9 and 12 hours) were the outcome variable and time in minutes from waking to the time that each cortisol sample was taken were the predictor variables (see data analysis section below).

Procedure

All participants were screened on the telephone in advance of being invited to visit the university. On arrival at the laboratory, each participant provided written consent. Participants completed the Self-Injurious Thoughts & Behaviors Interview (SITBI) with the researcher. Following the SITBI interview and risk assessment, participants completed a questionnaire pack which asked questions regarding their demographics and included a range of questionnaire measures.

Before leaving the laboratory, participants were instructed how to take cortisol salivary samples (see below) and provided with the kit containing everything they would need to take the required samples over the following 7 days. They were also provided with a copy of the study procedure prior to leaving the laboratory. All participants received an accelerometer (GeneActiv) device to wear on their wrist at all times for the following week. This was to improve adherence to the cortisol sampling protocol as the participants were aware that we were monitoring their wake and sleep times. The accelerometer was placed on participants' non-dominant hand.

For the next 7 days after their laboratory visit, participants completed an online diary to record daily stressors and emotions (outlined above) and a paper diary to record when each cortisol sample was due and the time the sample was taken. On their second visit to the laboratory, participants returned their cortisol samples, accelerometer and their saliva sampling diary (reporting the time samples were taken). They were then debriefed by the

researcher, and asked whether they had any questions regarding the study. Participants were reminded about the follow up interviews and informed that they would be contacted in approx. 1 month for the first follow up.

Follow-up interviews (at 1 month and 6 months) were conducted by telephone or online survey to assess levels of suicide ideation and suicide attempt status using the Self-Injurious Thoughts and Behaviors (SITBI) and the Beck Scale for Suicide ideation (BSSI).

Data analysis

The data were analyzed utilizing multi-level modeling using HLM 7 (Raudenbush et al., 2011) and the analysis was conducted in two stages for CAR and the WP-12 and then the rest of the day cortisol slope. Preliminary analyses showed that the attempt and ideation groups did not differ in terms of CAR ($p=0.37$) or WP-12 ($p=0.31$) but differed from the control group (ps <0.01). Therefore, in the main analyses, the attempt and ideation groups were combined into a single suicide vulnerability group and compared to the control group. The models for CAR and WP-12 were considered to have a two level hierarchical structure, with Level 1 capturing the within-person relations between the day-level predictors (daily stress and mood) and the day-level dependent variables (daily CAR, WP-12) and Level 2 capturing between-person variability (e.g., suicide vulnerability group). The Level 1 variables (daily stress and mood measures) were group mean centered (i.e., centered at individual level) and modelled as random as we assumed that each of the within-person variables would vary from day to day. The level 2 dichotomous variables (suicide vulnerability group, gender, medication usage, smoking status) were uncentered and Level 2 continuous variables were grand mean centered (age, BMI, childhood trauma). The level 2 variables were assumed to be fixed. Intraclass correlation coefficients (ICCs) are also provided for each of the models.

The data were analysed in three blocks. First, we examined whether suicide vulnerability group had cross-level (main) effects on daily CAR, daily WP-12 and cortisol levels across

each day (at 3, 6, 9 and 12 hours) and whether the within-person slope between sampling time and cortisol across the rest of the day was moderated by vulnerability group. Note, as outlined earlier, in the latter case, cortisol levels (at 3, 6, 9 and 12 hours) were the outcome variables and time in minutes from waking to the time that each cortisol sample was taken were the predictor variables. Second, we tested whether childhood trauma had cross-level (main) effects on daily CAR, daily WP-12 and cortisol levels across each day (at 3, 6, 9 and 12 hours) and whether the within-person slope between sampling time and cortisol across the rest of the day was moderated by childhood trauma. Third, we explored whether family history of suicide had any effects on the same cortisol variables. Note, in order to control for age, gender, body mass index (BMI), medication usage (i.e., reported using prescribed medication or not) and smoking status, these variables were treated as covariates and entered into all of the HLM models. In particular, we followed the recommendations put forward by Simmons, Nelson and Simonsohn (2011) in terms of transparency regarding the treatment of covariates. These authors have suggested that "if an analysis includes a covariate, authors must report the statistical results of the analysis without the covariate" (p.1363). Therefore, in order to strengthen the robustness of the current results, we present the main models first without any covariates and then with the covariates. In the main multi-level modelling analyses, in order to account for multiple testing, we have adopted a more conservative p-value in the final model ($p < 0.017$; $p < 0.05 / 3$) reflecting the tests conducted within each analysis block (e.g., the primary hypothesis tests the effects of suicide vulnerability group on three cortisol outcomes).

Multilevel mediation analysis using MPlus version 8 (Muthén & Muthén, 1998-2017) was performed to test whether the effects of childhood trauma on suicide vulnerability group status were mediated by daily cortisol levels. The same control variables were also entered into the MPlus models.

The general form of the cross-level (main effect) HLM model is expressed by the following equation:

$$\text{Daily CAR} = \beta_{00} + \beta_{01}\,(\text{age}) + \beta_{02}\,(\text{gender}) + \beta_{03}\,(\text{BMI}) + \beta_{04}\,(\text{medication}) + \beta_{05}\,(\text{Smoking}) + \beta_{06}\,(\text{vulnerability group})\, r_0 + \varepsilon$$

The general form of the "rest of day" cross-level model is expressed by the following equation:

$$\text{Rest of day cortisol levels} = \beta_{00} + \beta_{01}\,(\text{age}) + \beta_{02}\,(\text{gender}) + \beta_{03}\,(\text{BMI}) + \beta_{04}\,(\text{medication}) + \beta_{05}\,(\text{Smoking}) + \beta_{06}\,(\text{vulnerability group}) + \beta_{10}\,(\text{time}) + \beta_{11}\,(\text{vulnerability group} * \text{time}) + r_0 + r_1\,(\text{time}) + \varepsilon$$

When analysing the effects of daily stress and mood, the data were considered to have a three level hierarchical structure, Level 1 being the within-person slope between the time the sample was taken (predictor) and cortisol (outcome) levels (at 3, 6, 12 & 9 hours), Level 2 being within-person variation in daily stress or mood variables, and Level 3 being the between-person variability (e.g., childhood trauma, age). The influence of daily stress and mood variables were only investigated in relation to the rest of day cortisol measures as the former variables are unlikely to affect cortisol levels upon awakening on the same day.

The general form of the 3 level model is expressed by the following equation:

$$\text{Rest of day cortisol levels} = \beta_{000} + \beta_{001}\,(\text{age}) + \beta_{002}\,(\text{gender}) + \beta_{003}\,(\text{BMI}) + \beta_{004}\,(\text{medication}) + \beta_{005}\,(\text{Smoking}) + \beta_{006}\,(\text{vulnerability group}) + \beta_{010}\,(\text{daily stress}) + \beta_{011}$$

(daily stress * vulnerability group) + β_{100} (time) + β_{101} (vulnerability group * time) + $\varepsilon_0 + \varepsilon_1$(time) + r_{00} + r_{01}(daily stress) + r_{10} (time) + ε

In addition, in order to tease apart any within-person and between-person effects of our stress, mood, defeat and entrapment measures on cortisol levels, we also created person-level variables by averaging these across the 7 day time window. In these analyses, we entered the within-person variable (e.g., daily stress) at Level 1 together with the related between-person variable (e.g., person-level average of daily stress) at Level 2 and suicide vulnerability group at Level 3.

Hierarchical linear regression was utilized to test the final hypothesis following the procedures outlined by Kenny et al. (1998). First, in order to control for age, gender, BMI, medication usage and smoking status, adjusted cortisol values were calculated (in the form of creating residuals) by regressing the control variables against the 7 days mean values for AUCg, WP-12 and the rest of day cortisol levels. Second, for each outcome variable (suicide ideation at 1 month and at 6 months), study group (suicide attempt vs suicide ideation) was entered into model 1, baseline suicide ideation (and 1 month suicide ideation when predicting 6 month ideation) into model 2, adjusted AUCg, adjusted WP-12 or adjusted rest of day cortisol into model 3, and finally the study group by adjusted cortisol measure multiplicative interaction term entered into model 4. The multiplicative interaction term was entered to examine whether the effects of cortisol on future ideation/depression was different in the suicide attempt and ideation groups. Note the regression models followed the same format and non-significant predictors were retained in the models.

RESULTS

Descriptive statistics for the main study variables are presented in Table 2. Inspection of these data show that the mean levels of cortisol throughout the day were within acceptable normal ranges (Aardal and Holm, 1995; O'Connor et al., 2009). Moreover, the mean cortisol levels were higher in the control group compared to the ideation and attempt groups in the morning. As indicated earlier, the attempt and ideation groups exhibited similar levels of cortisol across the day. The daily stress and emotion variables were also lower in the control group compared to suicide vulnerability groups.

In terms of childhood trauma scores, individuals in the suicide attempt group scored significantly higher on all subscales of the CTQ measure compared to individuals in the control group, $F(10, 292)=6.98$, $p < 0.001$ (see Figure 1). The ideation group was intermediate to the two other groups on all the subscales, however, their scores were only significantly different from controls for physical neglect ($p<0.001$), emotional abuse ($p<0.001$), and for emotional neglect ($p<0.001$). In terms of exposure to any type of childhood trauma (that was moderate or severe), the highest levels of trauma were reported in the attempt group (79.2%), followed by the ideation (56.7%) and then the control (6.3%) groups.

Initial unconstrained models

Unconstrained models were run for each dependent variable in order to provide estimates of the within-person and between-person variance. For daily CAR the within-person and between-person variance coefficients were 0.26 and 0.22 (ICC = 0.48), respectively. For WP-12, the within-person and between-person variance coefficients were 0.09 and 0.04 (ICC = 0.13), respectively, Finally, for rest of day cortisol levels, the within-person and between-person variance coefficients were 0.10 and 0.04 (ICC = 0.14).

Effects of suicide vulnerability group on cortisol levels over 7 days

The findings for each model are presented in Table 3. The results for daily CAR showed that there was a main effect of suicide vulnerability group (0 = control, 1 = suicide vulnerability group) in the unadjusted (β = -0.269, p<.001; ICC = 0.45) and adjusted model (β = -0.283, p=0.002; ICC = 0.41), indicating that individuals in the suicide attempt and ideation groups secreted lower levels of cortisol upon waking. For the WP-12 measure, the effect of suicide vulnerability group was not significant in the unadjusted model (β = -0.072 p=0.09, ICC = 0.31), but was marginally significant (after Bonferroni correction) in the adjusted model (β = -0.098 p=0.026, ICC = 0.31) indicating that individuals in the suicide attempt and ideation groups had a tendency towards a flatter diurnal slope over 7 days compared to individuals in the control group (see Figure 2). As predicted, the level 1 slope between time and cortisol was significant in both the unadjusted (β = -0.136, p<0.001; ICC = 0.36) and adjusted models (β = -0.136, p<0.001; ICC = 0.36) indicating that cortisol levels declined across the day. However, there was no significant main effect of suicide vulnerability group on rest of day cortisol levels in the unadjusted (β = -0.003, p=0.938) or adjusted (β = -0.003, p=0.921) models and group did not moderate the level 1 sample time – cortisol relationship (unadjusted, β = -0.002, p=0.838; adjusted, β = -0.002, p=0.836).

Effects of childhood trauma on cortisol levels over 7 days

The findings for each model are presented in Table 4. The results for daily CAR showed that there was a main effect of childhood trauma in the unadjusted (β = -0.008, p=.015; ICC = 0.43) and adjusted model (β = -0.008, p=0.012; ICC = 0.40), indicating that individuals with higher levels of childhood trauma released lower levels of cortisol upon waking. For the WP-12 measure, there was a marginally significant effect (after Bonferroni correction) of childhood trauma in the unadjusted model (β = -0.002, p=.040; ICC = 0.31)

and a significant effect in the adjusted model (β = -0.003, p=0.007; ICC = 0.31), indicating that individuals with a history of trauma had a flatter diurnal slope during the day over 7 days compared to individuals with none-to-minimal childhood trauma, although, this effect was reliant on controlling for covariates (see Table 4, Figure 3). We repeated the analyses and entered each CTQ subscale separately in the HLM model (full results not shown). For daily CAR, we found that the effects of total childhood trauma were accounted for by significant effects of the emotional abuse (unadjusted β = -0.025, p=0.012, ICC = 0.43; adjusted β = -0.024, p=0.008, ICC = 0.41) and emotional neglect (unadjusted β = -0.021, p=0.009, ICC = 0.45; adjusted β = -0.02, p=0.004, ICC = 0.41) subscales. For WP-12, we found that the effects of total childhood trauma were accounted for by significant effects of the physical abuse subscale (unadjusted β = -0.015, p=0.003, ICC = 0.31; adjusted β = -0.016, p<0.001, ICC = 0.31) and less consistently so by the emotional neglect subscale (unadjusted β = -0.006, p=0.090, ICC = 0.31; adjusted β = -0.009, p=0.013, ICC = 0.31) given the latter findings were only observed after controlling for covariates.

Again, similar to suicide vulnerability group, there were no significant main effects of childhood trauma on rest of day cortisol levels (unadjusted, β = -0.002, p=0.938, ICC = 0.36; adjusted, β = -0.001, p=0.926, ICC = 0.36) and trauma did not moderate the Level 1 sample time – cortisol relationship (unadjusted, β = 0.001, p=0.612; adjusted, β = 0.001, p=0.605).

Indirect effects of childhood trauma on membership of suicide vulnerability group via daily cortisol levels

Next, using multilevel mediation analysis, we tested whether there were indirect effects of CTQ total score on membership of suicide vulnerability group (versus membership of control group) via daily CAR and WP-12 levels. In these analyses, total CTQ score (at Level 2) and suicide vulnerability group (at Level 2) were the X and Y variables,

respectively, and the daily CAR and WP-12 levels (at Level 1) acted as the mediators (M variables) in separate analyses. The analysis showed that there was an indirect effect of CTQ score on membership of the suicide vulnerability group through daily CAR levels (estimate = 0.001, p = 0.038; see Figure 4). There were also direct effects of childhood trauma (estimate = 0.009, p < 0.001) and daily CAR levels (estimate = -0.170, p = 0.029) on suicide vulnerability group, respectively. Taken together, these results show that higher levels of childhood trauma are associated with lower daily CAR levels that in turn are associated with a greater likelihood of being in the suicide vulnerable group. No indirect effects were found when WP-12 was considered to be a mediator; therefore, no further analyses were conducted.

In order to investigate whether specific types of childhood trauma had indirect effects on suicide vulnerability group membership via daily CAR levels further multilevel mediation analyses were conducted with the individual CTQ subscales. These results showed that daily CAR levels mediated the emotional abuse and suicide vulnerability group (estimate = 0.009, p = 0.033) and the emotional neglect and suicide vulnerability group (estimate = 0.040, p = 0.040) relationships.

Effects of daily stressors and emotions on the wake-peak to 12 hour slope and on rest of day cortisol levels

In order to explore whether daily stressors and emotions (including defeat and entrapment) had significant effects on diurnal cortisol levels, and whether any of these Level 1 slopes were moderated by suicidal vulnerability group, we ran a series of multi-level models for each stressor and emotion variable separately (while controlling for age, gender, BMI, medication use and smoking status). These analyses (not shown) found that none of the daily stressors or emotion variables were associated with the rest of day cortisol levels or

influenced the time of sample – cortisol relationship. In addition, suicide vulnerability group did not moderate any of the daily stress/mood – cortisol slopes.

As outlined earlier, we also explored the effects of the within-person daily stress/emotion variables on cortisol levels while including the person-level version of the daily stress/emotion variables in order to tease apart any potential within-person and between-person effects. However, none of the person-level variables was statistically significant and the results for within-person variables remained unchanged (data not shown).

Effects of family history

The results showed that there were no main effects of family history on daily CAR (unadjusted, β = -0.16, p=0.17, ICC = 0.46; adjusted, β = -0.06, p=0.62, ICC = 0.43) or on WP-12 (unadjusted, β = -0.05, p=0.34, ICC = 0.31; adjusted, β = -0.05, p=0.42, ICC = 0.31). In addition, there was no main effect of family history on rest of day cortisol (unadjusted, β = 0.01, p=0.80, ICC = 0.36; adjusted, β = 0.02, p=0.69, ICC = 0.36) and it did not moderate the time – cortisol slope (unadjusted, β = -0.01, p=0.77; adjusted, β = -0.01, p=0.77).

Predictive effects of mean cortisol levels on suicide ideation at 1 month and 6 months follow-up in suicide attempt and ideation groups

1-month follow-up

As outlined earlier, the predictive effects of daily cortisol levels (adjusted AUCg, WP-12 slope and rest of day levels) on suicide ideation at follow-up were examined using hierarchical regression. For suicide ideation at 1-month follow-up, study group (in model 1) did not significantly enter the equation. However, in model 2, baseline suicide ideation significantly explained 35% of the variance, F(1, 92)=50.92, p <0.001, such that higher levels of suicide ideation at baseline were associated with higher levels of ideation at 1 month

follow-up. Next, adjusted AUCg was entered into model 3 and significantly explained an additional 3% of the variance, $F(1, 91)=4.12$, $p = 0.045$. This result showed that individuals vulnerable to suicide who secreted lower cortisol levels at waking across the 7 day study reported higher suicide ideation one month later (whilst controlling for baseline suicide ideation and the other covariates). In model 4, the study group x adjusted AUCg interaction term did not significantly enter the equation. The regression analyses were repeated including adjusted WP-12 slope and rest of day levels in model 3 and the relevant interaction terms at step 4, however, these steps were not statistically significant.

6-month follow-up

For suicide ideation at 6-month follow-up, none of the adjusted mean cortisol level variables or their study group interaction terms significantly explained any additional variability at 6-month follow-up (results not shown).

Suicide attempt at 1-month and 6-month follow-up

One participant reported a suicide attempt at 1-month follow-up and additional attempt at 6 month follow-up. Another participant reported a suicide attempt at 6-month follow-up. Therefore, no further analyses were conducted.

DISCUSSION

This study represents the first 7 day daily diary investigation of naturally fluctuating cortisol levels in individuals vulnerable to suicide. The findings that individuals at risk of suicide showed a lower CAR and a tendency towards flatter diurnal cortisol slope compared to control participants is an important observation and is in keeping with a growing body of literature implicating HPA axis dysregulation in suicide vulnerability. For example, a recent study by Keilp et al. (2016) found evidence of low baseline cortisol levels in a suicide attempt group compared to a non-attempt group (Keilp *et al*., 2016) and an earlier

investigation also showed that low cortisol activity is associated with suicidal behavior (Lindqvist *et al*., 2008). More recently, Melham et al. (2017) reported that individuals with a suicide attempt history had lower hair cortisol concentrations compared to controls (as well as an ideation group). In terms of cortisol reactivity to stress, evidence is also converging to suggest dysregulation of the HPA axis, such that individuals vulnerable to suicide have an impaired stress response. Melhem et al. (2016) found the lowest levels of total cortisol output during a stressor in a sample of individuals (who were offspring of parents with mood disorder) with a suicide attempt history compared to an offspring group with suicide-related behavior but no suicide attempt history. Similarly, O'Connor et al. (2017) also found that individuals who had made a previous suicide attempt exhibited significantly lower cortisol response to an acute stressor compared to individuals in an ideation and a control group.

We also found high levels of childhood trauma in suicide vulnerable participants compared to control participants (and that childhood trauma was associated with lower CAR and flatter diurnal cortisol slope). In particular, we found that 79.2% of participants in the attempt group reported exposure to at least one type of childhood trauma that was classified as moderate to severe compared to 56.7% in the ideation group and 6.3% in the control group. The levels of trauma identified in the attempt and ideation groups are alarming, and in the former case, replicate the findings of an earlier investigation, whereby 78.7% of participants in an attempt group reported at least one type of moderate to severe childhood trauma (O'Connor et al., 2018). The relatively high levels of childhood trauma observed in the ideation group may explain why the attempt and ideation groups did not differ significantly in terms of their cortisol profiles. As we have indicated previously, such high levels of childhood trauma in suicide vulnerable groups have been reported in previous studies (e.g., Enns et al., 2006; Hassan, Stuart & De Luca, 2016; Marshall et al., 2013; Sachiapone et al., 2007). However, the current study extends the literature by showing that

the effects of childhood trauma (in particular, emotional abuse, emotional neglect) had indirect, as well as, direct effects on suicide vulnerability group membership through lower levels of daily CAR.

Moreover, we believe the current findings are important as they suggest that the experience of childhood trauma may predispose individuals to vulnerability to suicide in adulthood by leading to diminished HPA axis activity during awakening (and possibly a tendency towards a flatter diurnal profile across the day) as well as during stress (cf., O'Connor et al., 2018). A growing body of work is accruing to suggest that repeated activation of the HPA axis leads to dysregulation (e.g., Hwang et al., 2014; McEwen, 1998; 2000; Miller et al., 2007; O'Connor et al., 2018). This is known as allostatic load (McEwen, 1998), whereby if the HPA axis is repeatedly activated (for example, by chronic stress and exposure to childhood trauma) the immune, cardiovascular and the endocrine systems are potentially exposed to excessive demands that over time can lead to dysregulation of these systems (McEwen, 1998; 2000). In terms of the CAR (as well as reactivity to stress), there has been much debate about whether dysregulation related to exposure to chronic stress leads to hypercortisolism (enhanced secretion) or hypocortisolism (blunted secretion; Bernard et al., 2017; Miller et al., 2007; O'Connor et al., 2009; 2016; Segerstrom and Miller, 2004). Chida and Steptoe (2009) argue that in cases of fatigue, burnout and PTSD, it is possible that the mechanisms underlying the CAR become exhausted, like other cases of hypocortisolism. Empirical evidence is now converging to suggest that repetitive and sustained activation of the HPA axis stress system causes a blunted or reduced cortisol response over time (Fries et al., 2005; Hwang et al., 2014; O'Connor et al., 2009; 2013). This hypothesis is also in line with Fries et al's. (2005) account of the development of hypocortisolism, which suggests that the hypocortisolism occurs after a prolonged period of hyperactivity of the HPA axis due to chronic stress. Therefore, it is likely that in individuals who have experienced greater levels

of childhood trauma, over a more sustained period, their HPA axis may have become dysregulated leading to lower levels of CAR and a tendency towards a flatter slope across the rest of the day. Moreover, the current findings also suggest that the indirect effects of childhood trauma on lower daily CAR are associated with a greater likelihood of being vulnerable to suicide in adulthood.

We have previously argued that Lovallo's (2013) conceptual model of addiction linking adverse life experiences in childhood and adolescence to adverse health outcomes in adulthood can be usefully extended to suicide risk (O'Connor et al., 2018). Specifically, Lovallo (2013) contends that adverse life experiences cause modifications in frontolimbic brain function which may then lead directly to: 1) reduced stress reactivity, 2) altered cognition (characterised by a shift in focus to more short-term goals and impulsive response selection) and 3) unstable affect regulation. Furthermore, he argues that these three negative consequences influence the development of a more impulsive behavioral style that may increase risk of addiction and the engagement in poor health behaviours. We contend that exhibiting a low or blunted CAR may be another negative consequence of the modification of brain function (cf., Boehringer et al., 2015). Therefore, we would suggest that the development of a more impulsive behavioral style, and each of the precursors outlined above, including having a lower CAR, are associated with suicidal behavior. However, the causal mechanisms linking childhood trauma, lower CAR and suicide risk remain unclear. Several potential mechanisms have been suggested such as impaired executive function, poorer working memory, greater impulsive behaviors and less stable mood regulation (e.g., Lovallo, 2013; McGirr et al., 2010; O'Connor et al., 2017). These aforementioned mechanisms may also help explain our findings that lower CAR predicted increased levels of suicidal ideation at one-month follow-up in individuals vulnerable to suicide (after controlling for baseline levels and a full range of covariates) as well as the indirect effects on suicide vulnerability

group membership. Future research ought to focus on conducting investigations of these potential explanatory mechanistic variables under carefully controlled conditions as well as in naturalistic settings.

There are a number of limitations to the current study. First, the sample size could be considered small compared to large scale, epidemiological studies of suicide. However, in terms of experimental research in this area, this sample size is relatively large and also includes all the strengths of adopting a within-participants, daily diary design (e.g., multiple observations, using each participant as their own control etc.). Second, we acknowledge that the CTQ – our measure of childhood trauma – is a retrospective self-report tool that may be influenced by social (un)desirability, repression and memory biases. However, we note that scores on the CTQ are in the predicted direction (e.g., lowest in the control group and highest in the suicide attempt group) and, that if anything, retrospective self-report tools may be associated with an underestimation of actual occurrence (Hardt & Rutter, 2004). Third, although it may have been useful to assess clinical diagnoses of psychiatric disorders in this study, our approach is consistent with the recent attention on the research domain criteria (Glenn, Cha, Kleiman, & Nock, 2017) and focus on dimensions rather than psychiatric classifications. Therefore, future research ought to collect more detailed, formal information on current psychiatric diagnoses, as well as lifetime history of psychiatric and psychological disorders. Fourth, we recognise that the current study did not include an objective test of participant adherence to the cortisol sampling protocol such as electronic containers for Salivettes which record the time at which they were opened. These containers are costly and would have been prohibitively expensive to include in the current study given the large number of samples per participant (n=56). Nevertheless, we included a number of methodological features to the study that are likely to have substantially reduced protocol adherence problems (e.g., participants wore an accelerometer to record wake time, we

explained that the experimenters could identify protocol non-adherence in the sampling, we ensured that participants kept diaries and received reminders).

In conclusion, this is the first 7-day daily diary investigation of naturally fluctuating cortisol levels in individuals vulnerable to suicide. The results extend other findings from the laboratory into naturalistic settings and indicate that dysregulated hypothalamic-pituitary-adrenal (HPA) axis activity is associated with suicidal behaviour. Childhood trauma appears to be an important distal factor associated with the development of HPA axis dysregulation. The challenge for researchers is to elucidate the precise causal mechanisms linking trauma, cortisol and suicide risk in order to develop interventions to help build resilience in vulnerable populations.

Footnote:

1. The cortisol awakening response can also be operationalised as Area Under the Curve with respect to increase (AUCi). Therefore, note that in separate unadjusted and adjusted (for covariate) analyses, we tested whether there were any effects of suicide vulnerability group on AUCi. No significant effects were found for the unadjusted model (β = - 1.15, p=0.23) and the adjusted model (β = -0.63, p=0.55).

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Table 1. Baseline characteristics for participants in each study group (n = 142)

Characteristic	**Control group** (n=47)	**Ideation group** (n=46)	**Attempt group** (n=49)
Age (SD)	25.74 (6.8)	27.72 (10.4)	30.28 (10.15)
Sex (% female)	33 (70.2)	26 (56.5)	40 (81.6)
Ethnic background (%)			
White British	32 (68.1)	27 (58.7)	29 (59.2)
White Irish	1 (2.1)	2 (4.3)	1 (2.0)
White European	1 (2.1)	3 (6.5)	2 (4.1)
Other White background	1 (2.1)	3 (6.5)	2 (4.1)
Other mixed/multiple background	1 (2.1)	1 (2.2)	1 (2.0)
White and Asian		2 (4.3)	
Indian	4 (8.5)	1 (2.2)	1 (2.0)
Pakistani			2 (4.1)
Chinese		3 (6.5)	1 (2.0)
Other Asian	5 (10.6)	2 (4.3)	2 (4.1)
African	1 (2.1)	2 (4.3)	6 (12.2)
Caribbean	1 (2.1)		2 (4.1)
Medication status[±]	6	17	22
Smoking status	4	7	17
Current psychiatric/psychological diagnosis*			
Depression	0	10	14
Anxiety	0	6	5
Bipolar disorder	0	0	1
Post-traumatic stress disorder	0	1	1
Number of lifetime attempts[+]			1 attempt = 24 2 attempts = 8 3 attempts = 7 4 attempts = 2 ≥ 5 attempts = 8
Method in most recent attempt[+]			
Own prescription drugs			30
Illicit drugs (not rx)			1
Over-counter drugs			8
Firearm			1
Immolation			1
Hanging			4
Sharp object			1
Auto exhaust			1
Train/car			1
Drowning			1
Family history of suicide (%)	4 (8.5)	8 (17.5)	13 (26.5)
Prescribed medications (%)	6 (12.8)	17 (36.9)	22 (44.9)

* = Participants were asked to provide details of any current diagnosed medical conditions; physical and/or psychiatric/psychological; [±] = reported using prescribed medications, [+] = From Self-Injurious Thoughts and Behaviors Interview

Table 2. Descriptive statistics (means and standard deviations) for main study variables in control, ideation and attempt groups (n = 142)

	Control group (n=47)		Ideation group (n=46)		Attempt group (n=49)		Combined attempt & ideation gp	
	M	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***
Cortisol variables								
Waking (00 min) (nmol/L)	8.35	3.84	7.51	3.45	7.16	4.06	7.34	3.76
15 min (nmol/L)	9.87	4.09	9.16	3.98	8.56	4.53	8.86	4.26
30 min (nmol/L)	11.46	4.62	10.02	4.34	9.63	4.88	9.83	4.61
45 min (nmol/L)	11.23	5.13	9.06	4.37	9.12	4.63	9.09	4.50
3 hr (nmol/L)	4.47	2.25	4.42	2.54	4.58	2.67	4.50	2.61
6 hr (nmol/L)	3.55	2.08	3.52	2.19	3.52	2.08	3.52	2.14
9 hr (nmol/L)	2.42	1.59	2.48	1.60	2.73	1.89	2.61	1.75
12 hr (nmol/L)	1.80	1.55	1.77	1.63	1.83	1.41	1.80	1.52
CAR (nmol/L)	31.12	10.63	27.47	10.32	26.33	11.98	26.90	11.15
Wake-peak to 12 hr (nmol/L)	11.50	4.90	9.79	4.48	9.45	4.80	9.62	4.64
Total no. of daily stressors	1.84	1.50	2.26	1.78	2.10	1.72	2.18	1.75
Daily perceived stress	9.13	3.28	11.22	3.50	11.63	3.82	11.43	3.66
Negative mood	9.00	4.53	10.82	4.84	11.63	6.11	11.23	5.48
Positive mood	13.52	4.70	12.34	4.99	10.48	4.28	11.41	4.64
Defeat	1.45	0.89	1.99	1.20	2.33	1.44	2.16	1.32
Entrapment	1.33	0.78	2.12	1.31	2.35	1.46	2.24	1.39
Between-person variables								
Total CTQ score	31.36	5.40	43.37	13.31	54.00	21.03	48.69	17.17
Physical neglect	5.53	0.97	7.91	3.47	8.55	4.09	8.23	3.78
Emotional abuse	6.91	2.30	10.87	5.31	13.51	6.31	12.19	5.81
Emotional neglect	8.36	3.11	12.35	4.99	14.41	5.09	13.38	5.04
Physical abuse	5.51	1.04	5.83	1.52	8.71	5.35	7.27	3.43
Sexual abuse	5.04	0.29	6.41	4.01	8.82	6.71	7.62	5.36
Suicidal ideation-baseline	0.00	0.00	0.57	0.92	0.94	1.18	0.75	1.05
Suicidal ideation-1 month	0.00	0.00	0.56	0.97	0.62	0.92	0.59	0.95
Suicidal ideation-6 months	0.03	0.16	0.80	0.95	1.00	1.35	0.90	1.15

Note: Cortisol awakening response (CAR) is measured using area under the curve with respect to ground (AUCg); Total CTQ = total Childhood Trauma Questionnaire score

Table 3. Effects of suicide vulnerability group on cortisol awakening response (CAR), wake-peak to 12 hours and rest of day diurnal cortisol levels across 7 days in suicide vulnerability group (n = 95) versus control group (n = 47)

	Unadjusted					Adjusted for covariates				
		Coeff	**SE**	***d.f.***	***P* value**		**Coeff**	**SE**	***d.f.***	***P* value**
Daily CAR										
Intercept	β_{00}	3.180	0.112	140	<0.001	β_{00}	3.174	0.184	135	<0.001
Vulnerability group	β_{01}	-0.269	0.076	140	<0.001	β_{01}	-0.283	0.088	135	0.002
Age	β_{02}	--	--	--	--	β_{02}	0.015	0.003	135	<0.001
Gender	β_{03}	--	--	--	--	β_{03}	0.029	0.101	135	0.770
BMI	β_{04}	--	--	--	--	β_{05}	-0.013	0.008	135	0.107
Medication status	β_{05}	--	--	--	--	β_{06}	0.054	0.087	135	0.499
Smoker status	β_{06}	--	--	--	--	β_{07}	-0.198	0.123	135	0.110
Wake-peak to 12 hours										
Intercept	β_{00}	1.016	0.072	140	<0.001	β_{00}	1.064	0.109	135	<0.001
Vulnerability group	β_{01}	-0.072	0.042	140	0.090	β_{01}	-0.098	0.043	135	0.026
Age	β_{02}	--	--	--	--	β_{02}	0.004	0.002	135	0.018
Gender	β_{03}	--	--	--	--	β_{03}	-0.007	0.041	135	0.856
BMI	β_{04}	--	--	--	--	β_{05}	0.005	0.003	135	0.155
Medication status	β_{05}	--	--	--	--	β_{06}	0.042	0.044	135	0.343
Smoker status	β_{06}	--	--	--	--	β_{07}	-0.027	0.054	135	0.619
Rest of day										
Intercept	β_{00}	0.413	0.053	140	<0.001	β_{00}	0.337	0.070	135	<0.001
Vulnerability group	β_{01}	-0.002	0.033	140	0.938	β_{01}	-0.003	0.035	135	0.921
Age	β_{02}	--	--	--	--	β_{02}	0.001	0.001	135	0.264
Gender	β_{03}	--	--	--	--	β_{03}	0.045	0.034	135	0.184
BMI	β_{04}	--	--	--	--	β_{04}	-0.003	0.003	135	0.276
Medication status	β_{05}	--	--	--	--	β_{05}	0.002	0.031	135	0.935
Smoker status	β_{06}	--	--	--	--	β_{06}	-0.004	0.036	135	0.896
Level 1 slope										
Time – cortisol levels	β_{10}	-0.136	0.022	140	<0.001	β_{10}	-0.136	0.022	140	<0.001
Group * time – cortisol levels	β_{11}	-0.002	0.012	140	0.838	β_{11}	-0.002	0.012	140	0.838

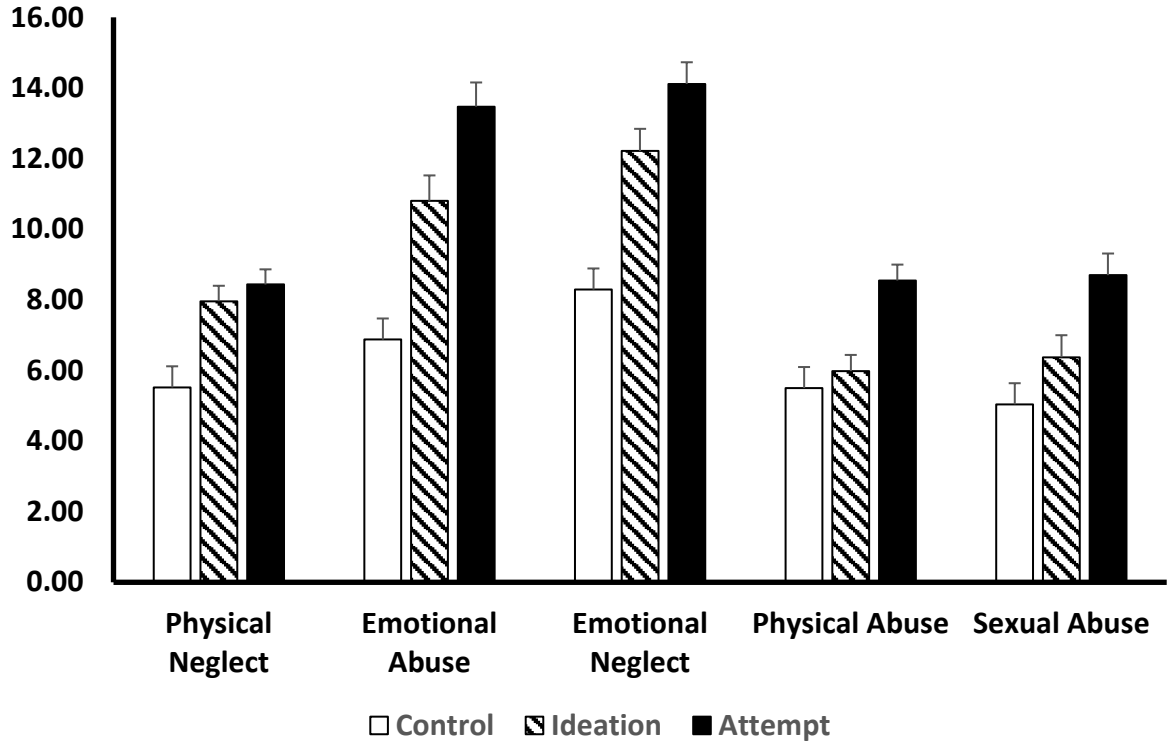
Note: CAR is measured using area under the curve with respect to ground (AUCg)

Table 4. Effects of childhood trauma on the cortisol awakening response (CAR), wake-peak to 12 hours and rest of day diurnal cortisol levels across 7 days in suicide vulnerability group (n = 95) versus control group (n = 47)

	Unadjusted					Adjusted for covariates				
		Coeff	**SE**	***d.f.***	***P* value**		**Coeff**	**SE**	***d.f.***	***P* value**
Daily CAR										
Intercept	β_{00}	2.723	0.042	140	<0.001	β_{00}	2.713	0.184	135	<0.001
Childhood trauma	β_{01}	-0.008	0.004	140	0.018	β_{01}	-0.008	0.003	135	0.012
Age	β_{02}	--	--	--	--	β_{02}	0.016	0.003	135	<0.001
Gender	β_{03}	--	--	--	--	β_{03}	0.026	0.096	135	0.786
BMI	β_{04}	--	--	--	--	β_{05}	-0.012	0.008	135	0.121
Medication status	β_{05}	--	--	--	--	β_{06}	-0.008	0.076	135	0.909
Smoker status	β_{06}	--	--	--	--	β_{07}	-0.142	0.100	135	0.162
Wake-peak to 12 hours										
Intercept	β_{00}	0.895	0.020	140	<0.001	β_{00}	0.903	0.072	135	<0.001
Childhood trauma	β_{01}	-0.002	0.001	140	0.040	β_{01}	-0.003	0.001	135	0.007
Age	β_{02}	--	--	--	--	β_{02}	0.005	0.002	135	0.013
Gender	β_{03}	--	--	--	--	β_{03}	-0.008	0.041	135	0.845
BMI	β_{04}	--	--	--	--	β_{05}	0.005	0.004	135	0.165
Medication status	β_{05}	--	--	--	--	β_{06}	0.020	0.043	135	0.641
Smoker status	β_{06}	--	--	--	--	β_{07}	-0.007	0.057	135	0.900
Rest of day										
Intercept	β_{00}	0.413	0.053	140	<0.001	β_{00}	0.330	0.065	135	<0.001
Childhood trauma	β_{01}	-0.002	0.033	140	0.938	β_{01}	-0.001	0.001	135	0.926
Age	β_{02}	--	--	--	--	β_{02}	0.001	0.001	135	0.241
Gender	β_{03}	--	--	--	--	β_{03}	0.045	0.034	135	0.186
BMI	β_{04}	--	--	--	--	β_{04}	-0.003	0.003	135	0.284
Medication status	β_{05}	--	--	--	--	β_{05}	0.003	0.029	135	0.893
Smoker status	β_{06}	--	--	--	--	β_{06}	0.001	0.038	135	0.976
Level 1 slope										
Time – cortisol levels	β_{10}	-0.141	0.006	140	<0.001	β_{10}	-0.140	0.005	140	<0.001
Trauma * time – cortisol levels	β_{11}	0.001	0.001	140	0.612	β_{11}	0.001	0.001	140	0.605

Note: CAR is measured using area under the curve with respect to ground (AUCg)

Figure 1. Childhood trauma scores (upper panel) and total exposure to any type of "Moderate to Severe" childhood trauma (lower panel) in attempt, ideation and control groups (error bars = SEM)



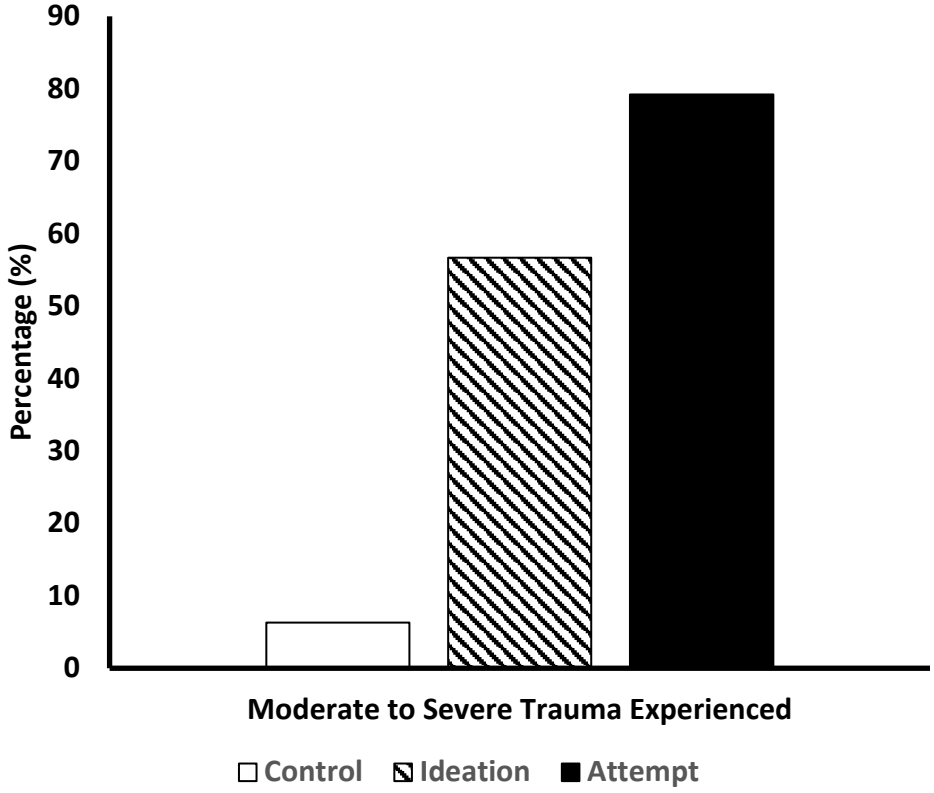


Figure 2: Effects of suicide vulnerability group on cortisol awakening response (CAR) and wake-peak to 12 hours slope (n=142) (error bars = SEM)

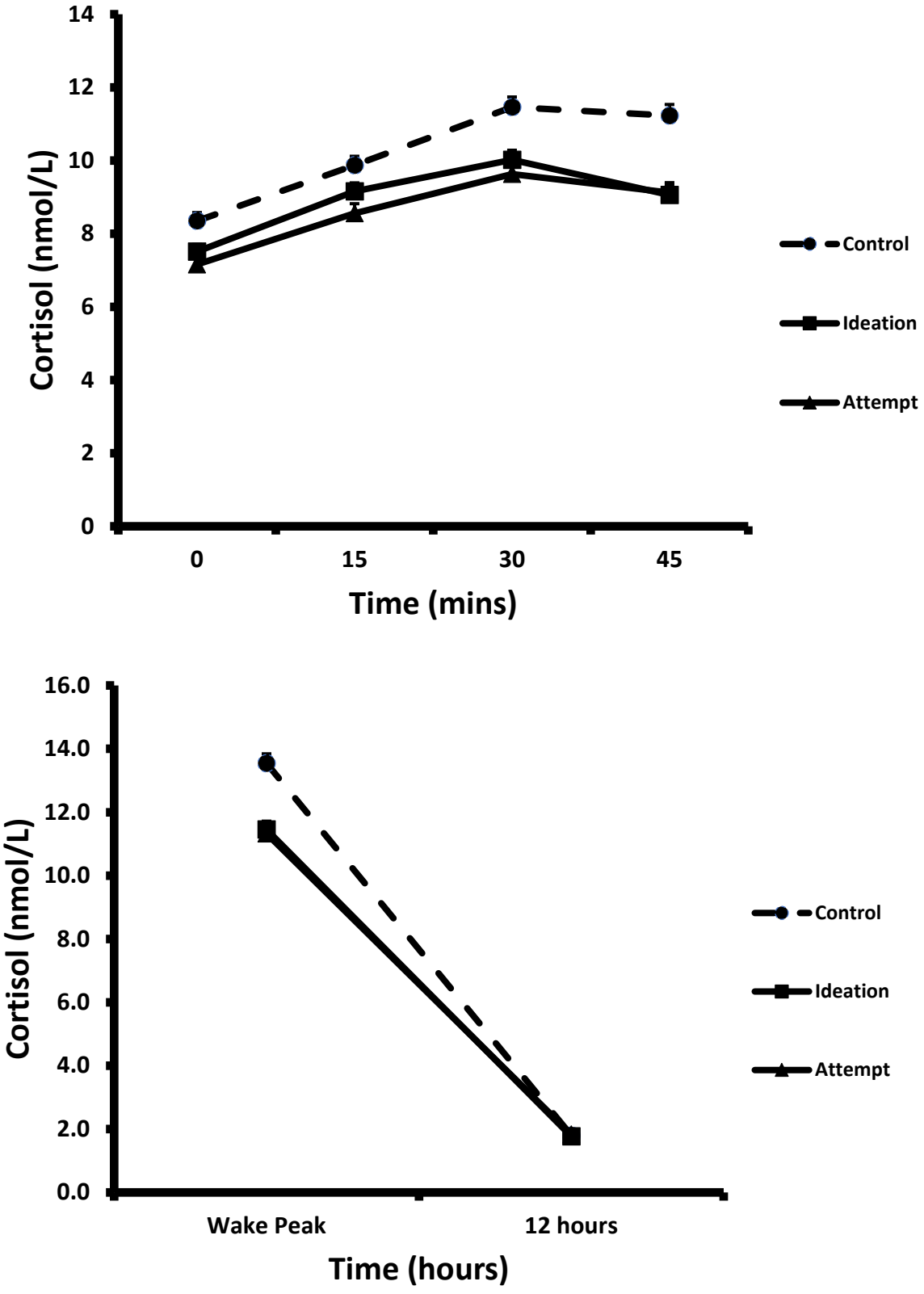


Figure 3: Effects of childhood trauma levels on cortisol awakening response (CAR) and wake-peak to 12 hours slope (n=142) (error bars = SEM)

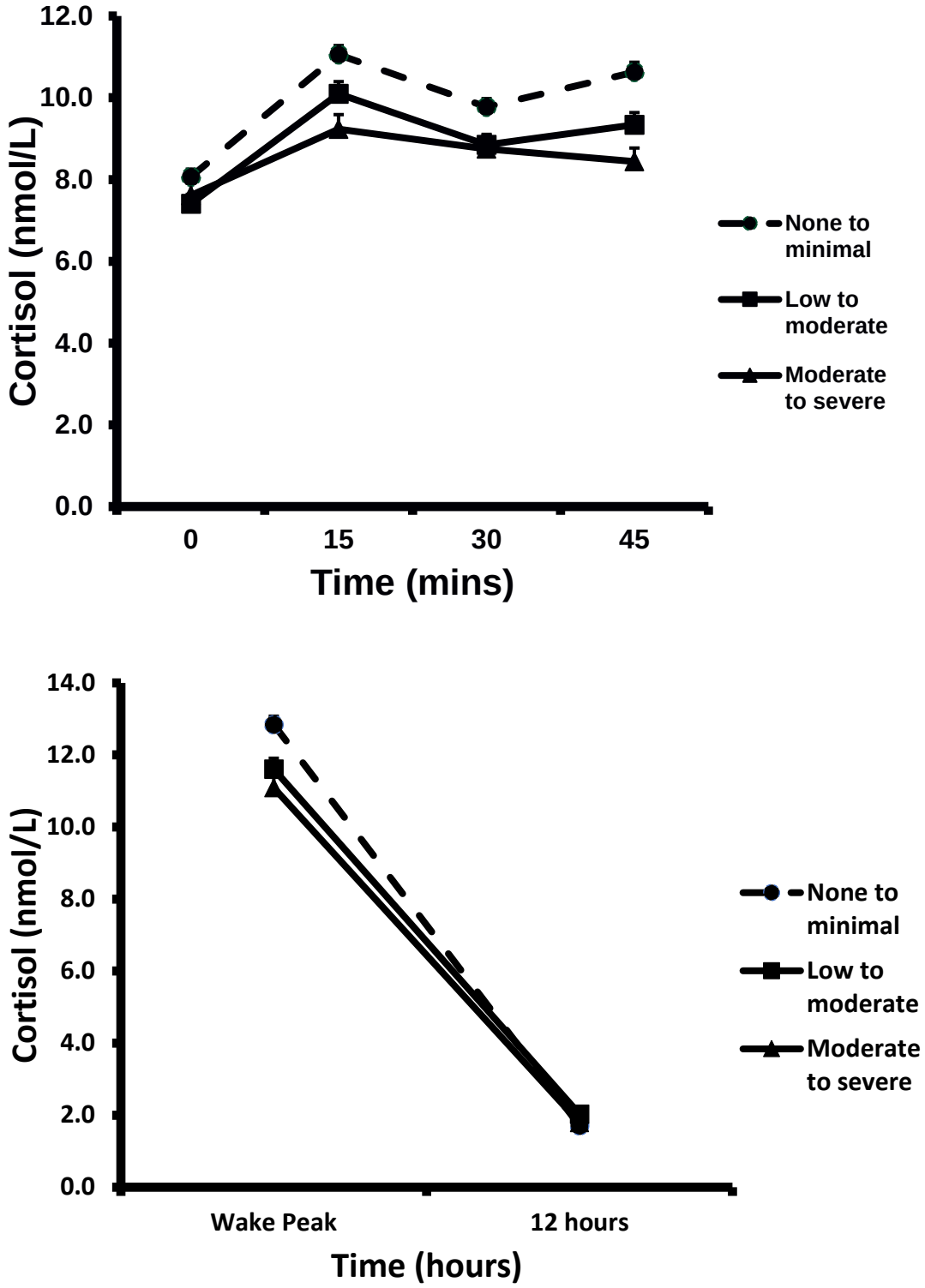
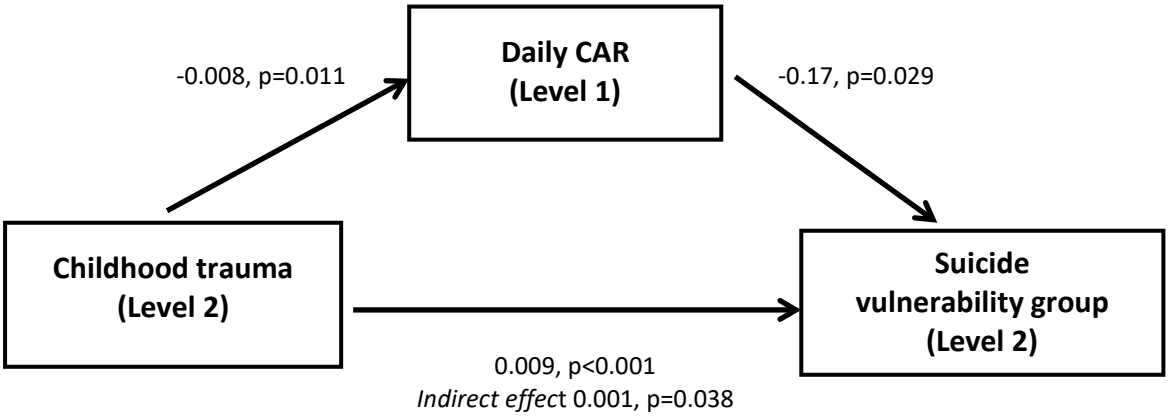


Figure 4: Indirect effect of childhood trauma on suicide vulnerability group membership via (lower) daily cortisol awakening response (CAR) levels



Supplementary Table 1. Effects of daily stressor and emotions on rest of day diurnal cortisol levels across 7 days in suicide vulnerability group (n = 95) versus control group (n = 47)

	Unadjusted					Adjusted for covariates				
		Coeff	**SE**	***d.f.***	***P* value**		**Coeff**	**SE**	***d.f.***	***P* value**
Total stress										
Intercept	β_{000}	0.630	0.048	140	<0.001	β_{00}	0.571	0.067	135	<0.001
Vulnerability group[1]	β_{001}	-0.013	0.028	140	0.618	β_{01}	-0.010	0.029	135	0.724
Age	β_{002}	--	--	--	--	β_{02}	0.001	0.001	135	0.611
Gender	β_{003}	--	--	--	--	β_{03}	0.033	0.027	135	0.224
BMI	β_{004}	--	--	--	--	β_{05}	-0.003	0.002	135	0.155
Medication status	β_{005}	--	--	--	--	β_{06}	0.008	0.028	135	0.978
Smoker status	β_{006}	--	--	--	--	β_{07}	-0.012	0.032	135	0.689
Slope										
Total stress – diurnal cortisol	β_{010}	-0.009	0.021	140	0.643	β_{010}	-0.009	0.021	140	0.645
Group * stress – diurnal cortisol	β_{011}	0.007	0.011	140	0.544	β_{011}	0.007	0.011	140	0.548
Slope[1]										
Time – diurnal cortisol	β_{100}	-0.152	0.020	140	<0.001	β_{100}	-0.152	0.020	140	<0.001
Group * time– diurnal cortisol	β_{101}	0.002	0.011	140	0.863	β_{101}	0.002	0.011	140	0.864
Perceived stress										
Slope										
Stress – diurnal cortisol	β_{010}	0.006	0.011	140	0.540	β_{010}	0.006	0.011	140	0.537
Group * stress – diurnal cortisol	β_{011}	-0.005	0.006	140	0.366	β_{011}	-0.005	0.006	140	0.365
Negative mood										
Slope										
Negative mood – diurnal cortisol	β_{010}	-0.004	0.007	140	0.585	β_{010}	-0.004	0.007	140	0.581
Group * mood – diurnal cortisol	β_{011}	0.002	0.004	140	0.568	β_{011}	0.002	0.004	140	0.564
Positive mood										
Slope										
Positive mood – diurnal cortisol	β_{010}	-0.007	0.006	140	0.314	β_{010}	-0.007	0.006	140	0.322
Group * mood – diurnal cortisol	β_{011}	0.005	0.004	140	0.174	β_{011}	0.005	0.004	140	0.178
Defeat										
Slope										

Defeat – diurnal cortisol	β_{010}	-0.019	0.031	140	0.540	β_{010}	-0.019	0.031	140	0.542
Group * defeat – diurnal cortisol	β_{011}	0.007	0.017	140	0.654	β_{011}	0.007	0.017	140	0.655
Entrapment										
Slope										
Entrapment – diurnal cortisol	β_{010}	-0.016	0.040	140	0.682	β_{010}	-0.016	0.040	140	0.683
Group * entrap – diurnal cortisol	β_{010}	0.010	0.021	140	0.632	β_{011}	0.010	0.021	140	0.633

Note: [1] = this block of results are the same for each model, therefore, they are not reported below for each daily stress and emotion variables